SPREAD OF INFECTION FROM THE RESPIRATORY TRACT OF THE FERRET. I. TRANSMISSION OF INFLUENZA A VIRUS.

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THERE is general recognition of the fact that infective agents may pass from the respiratory tract of one host to that of another through the air. There has, however, been but little study of the precise mechanism of this transmission under experimental conditions. The term " droplet infection " is in common use ; this includes infection caused by the scattering of fairly large drops from the mouth or nose. Wells and Wells (1936) have lately emphasized the importance of droplet nuclei : droplets which are fairly large as they leave the mouth are expelled with such speed that they rapidly lose fluid by evaporation until they become so small that they can float in the air for many hours. Wells and Wells have urged that for this reason " droplet nuclei " form a greater source of danger than the coarse particles. Infection may be carried in the air in yet a third way, on particles of dust. The relative importance of the various potential vehicles of infection is hard to determine under field conditions. We have therefore attempted to study the problem experimentally, deliberately avoiding the somewhat artificial conditions produced by spraying suspensions of organisms into the air. We began by investigating aerial transmission of influenza A virus in the ferret, as we know of no other infection more suitable for our purpose.

Experimental Methods.

Ferrets under investigation were housed in the experimental animal isolation hospital at Mill Hill. This contains 32 separate cubicles 6 ft. 9 in. by 5 ft. 9 in. by 9 ft. by 9 in. high, each provided with a window facing the door. Each experiment was carried out in a separate cubicle, and the walls and floors of each cubicle were washed down with lysol followed by water from a high-pressure hose between two experiments. For the experiments on direct contact ferrets were housed in special cages—metal-sided boxes 1 ft. 6 in. by 1 ft. 6 in. by 1 ft. 6 in. high, with perforated metal lids ; for other trials open wire-meshed rabbit cages were used. Attendants wore rubber boots and mackintoshes which were kept wet with lysol ; care was taken by dipping hands, tongs, thermometers and so forth in lysol to prevent mechanical transfer of infection from one animal to another.

Infection by Immediate Contact.

It is known from earlier work (Stuart Harris, Andrewes and Smith, 1938) that ferrets running together on the floor of a cubicle will transmit infection from one to another. We readily confirmed that close contact regularly permitted cross-infection, and also paid attention to the possibility that repeated transfer in this manner might modify the properties of the virus. A normal ferret was placed in a metal cage with one which had been infected 24 hours previously with a ferret-adapted strain of influenza A virus (WS). Four days later the originally infected ferret was removed and another clean one introduced. The second ferret duly sickened with influenza and proved capable of passing the infection to the third. In a similar manner the contact series was carried on through eight ferrets, the period of contact of normal and infected ferrets varying from two to four days. All ferrets developed fever of 104·6° F. or higher with typical nasal symptoms: no suggestion of modification of the virus was forthcoming.

When a normal and an infected ferret have been placed in adjacent metal cages infection has usually failed to pass from one to the other. We have not, however, relied in practice upon the negative results of the few experiments which were designed to test this point; experimental ferrets are kept, except for special reasons, in cages each in a separate cubicle.

Infection by Exposure at a Distance.

Experiments were next carried out to determine over what distance a ferret with influenza could project infected particles in sufficient numbers to convey the disease. Ferrets were placed in the isolation cubicles already mentioned and were housed in open wire-mesh rabbit cages on racks. In earlier experiments one infected or "donor" ferret was placed in each cubicle; later we used two "donors" and two normals or "recipients." The two donors were kept in one cage—a proceeding which apparently increased their activity. In order to decrease the chance of accidental transference of infection by human agency, temperatures of the donors only were taken, except in the earliest trials, until a typical febrile response occurred in them; thereafter recordings on the recipients were begun and confined to these. Table I shows the results of eleven experiments. A decision as to whether any given ferret had "taken" or not was based on the temperature response, the symptoms and the result of a subsequent immunity test to the same (WS) strain of virus. The last was particularly necessary, as many exposed ferrets developed a milder disease than that following intranasal inoculation of virus. It will be seen that, in three experiments, only one of two recipients contracted the disease; this indicates that in such experiments the amount of virus passing across the cubicle must have been near the threshold dose. Further, in experiments 2 and 4, one recipient became infected three days after the other and may have caught the disease from his neighbour rather than from the donors. Very probably cross-infection from one recipient to the other

would have occurred also in experiments 3, 8 and 10 if their contact had been longer continued.

Table I shows that with one donor ferret in a cubicle, infection of one or both recipients occurred in two out of three trials ; with two donors present, six out of eight experiments were positive. Transmission took place in seven tests over an air-distance of 5 ft. ; longer distances could not be tested conveniently in the cubicles available. In order to diminish the chances of effective projection of coarse droplets by a sneezing ferret, recipients were in most trials placed at a higher level than donors ; there is evidence that the larger particles settle before they have travelled more than 1 or 2 ft., though we cannot say that the infection of a normal ferret placed 5 ft. away from and 3 ft. above an infected ferret *must* have been due to particles below a given size. At any rate the higher level of a recipient's cage did not apparently interfere with the likelihood of cross-infection.

TABLE I.—*Infection of Ferrets across a Cubicle.*

Experiment.	No. of "donors."	Distance between ferrets. Total.	Distance between ferrets. Vertical.	Influenza "take" in recipients.	Incubation period in days.
1	2	5 ft.	Recipient 2 ft. 10 in. above donor	+	5
		2 ft. 3 in.	Level	+	5
2	1	2 ft. 3 in.	Level	+	11
		5 ft. 6 in.	Recipient 2 ft. 10 in. above donor	+	8
3	1	3 ft. 3 in.	Recipient 3 ft. above donor	+	7
		5 ft.	Recipient 3 ft. 6 in. above donor	0	–
4	2	,,	3 ft.	+	5
				+	8
5	2	,,	,,	+	3
				+	3
6	2	,,	,,	+	4
				+	4
7	1	,,	,,	0	–
8	2	,,	,,	+	6
				0	–
9	2 ; only one "took"	,,	,,	0	–
				0	–
10	2	,,	,,	? 0	–
				+	6
11	2	,,	,,	0	–
				0	–

Experiments with Screens.

In order to test whether infection was caused by droplets sneezed directly from one ferret to another, we interposed light plywood screens 4 ft. 6 in. by 2 ft. 6 in. in the centre of the cubicle so as to shield recipients from droplets directly projected by the infected ferrets. The screen had an opening at the base 1 ft. square, with its centre 1 ft. from the floor. A fan was fitted on the side nearest the recipient so that a current of air was blown towards the donor at a rate of about 20 ft. a second. It would then have to travel up past the donor and over the top of the screen in order to carry particles to the recipient. In two experiments, each with two donors and two recipients, no transmission of infection occurred, despite severe infection and repeated sneezing on the part of the donors. The success of our barrier against direct projection might be explained on the grounds that the coarser droplets are of major importance. Alternatively, one could argue that, since successful transmission did not occur in 100 per cent. of the control experiments, anything interfering with the numbers of infected particles in the air might lower the chances of success.

Experiments with Good Ventilation.

Tests on the rate of disappearance of CO_2 liberated into a cubicle showed that there was a very low air turnover when the doors and windows were shut. Increased ventilation of a cubicle might be expected to dilute the "infective" particles in the air and interfere with cross-infection, more especially, perhaps, if direct projectiles were not of prime importance. In two trials, each with two donors and two recipients, the windows in the cubicles were left wide open. The clean ferrets were placed near the bottom of the windows, and the current of air flowed from them towards the infected animals, then upwards and out of the window again at a higher level. In one of the trials the natural air currents were assisted by a fan placed near the donors. In neither experiment did any cross-infection occur, despite seven days' exposure of the normal ferrets to the infected ones.

Experiments with Ducts.

In order that factors, such as air currents, which could not be adequately controlled in a cubicle, might cease to act as possible sources of error, closed ducts were constructed of light wood, as shown in Fig. 1. The dimensions of the ducts as seen from above are indicated in the diagram; they were almost square in cross-section, $10\frac{1}{2}$ in. wide and $9\frac{1}{2}$ in. high, leaving room for small open-meshed wire cages 1 ft. 1 in. by $8\frac{1}{2}$ in. by 8 in. in height, one at each end. Access to the cages was secured by tightly fitting glass panels in the roof over each cage. The end-plate next to the donor ferret was perforated by 13 round holes, each 1 in. in diameter; the corresponding plate opposite the side of the recipient ferret's cage was similarly perforated, but with 13 holes, each $\frac{1}{4}$ in. in diameter. This arrangement was designed to ensure a uniform flow of air at a low speed in the same direction. Just outside the latter was a

four-blade electrically-driven extraction fan with a variable rheostat. As the figure shows, three ducts were built—straight, S-shaped and U-shaped respectively.

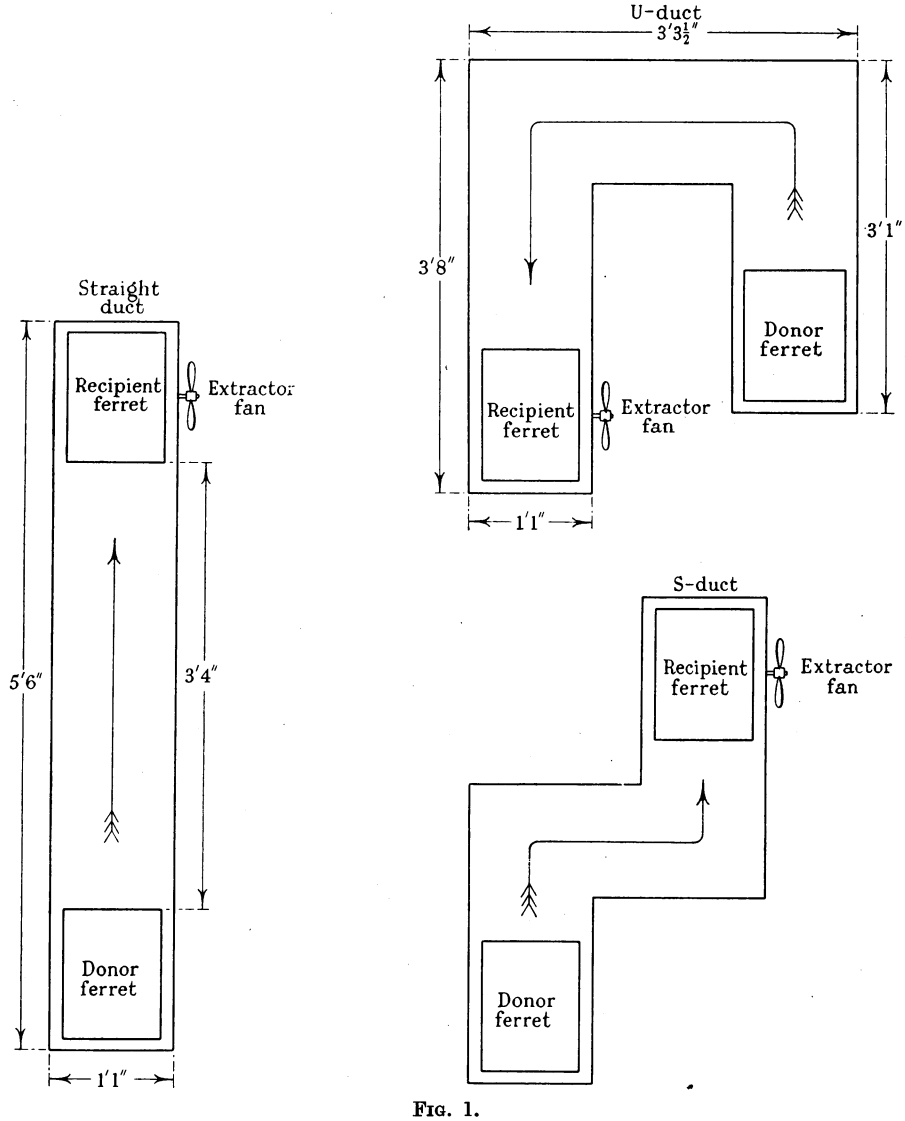

FIG. 1.

Two normal ferrets were first infected to act as donors. When fever and symptoms were in evidence after 48 hours they were placed, one each, in the donor cages of the straight and S-ducts and normal ferrets were put into the recipient cages. The fans were adjusted to draw through 14 linear feet of air a minute. Temperatures on the recipients were taken twice daily, particular

care being taken to avoid accidental contamination. Thus, before the ferrets were handled for any purpose, the fans were stopped and the animal concerned with its cage removed from the cubicle. In two further experiments (Exp. 13 and 14) the U-shaped duct was compared with the straight duct. The results are shown in Table II.

TABLE II.—*Experiments with Ducts.*

Experiment No.	No. of recipient ferret.	Duct used.	ft./min. Air-speed	Result.	Incubation period in days.
12	129	Straight	14	+	6 (?)
	130	S—	14	+	3
13	159	Straight	3	Mild take, immune later	5
	160	U—	3	Do.	7
14	177	Straight	13	+	8
	178	U—	13	+	6

From Exp. 12 it appeared that infection had occurred through the S-duct. It was thought likely that at the low air-speed employed coarse particles would not be swept round the bends, but would impinge upon the walls and be trapped. We soon perceived, however, that a U-shaped duct would test the point at issue more satisfactorily. As Table II shows, infection occurred round the bend of the U, both at the low and very low air-speeds. It seems highly improbable that droplets of the order of 0·1 to 1 mm. in diameter could be carried round corners at such low speeds, and the inference seems natural that infection was conveyed either by droplet-nuclei or very fine dust particles.

DISCUSSION.

There are several indications from the above experiments that the infection-bearing particles in influenza A of ferrets are not exclusively the gross droplets which are expelled when the animal sneezes. Infection occurs over a distance of over 5 ft. in almost still air; it can even travel upwards and infect a normal ferret in a cage several feet above an infected animal. Good ventilation, which would be expected to decrease the numbers of infective particles, seems to interfere with the chances of infection. Trials in ducts indicate that the particles are small enough to be wafted round right-angle bends by air-currents as sluggish as 3 ft. a minute. On the other hand, the importance of direct projectiles is suggested by the fact that screens interposed to shield recipient ferrets from direct droplets did seem to have value in stopping cross-infection. Unfortunately in control experiments infection across a cubicle did not take place with complete regularity; we cannot afford to lay as much weight on negative evidence, such as that provided by the screen and ventilation experiments, as on the positive results of others and of the duct trials.

Our experiments do not deny that coarse droplets may convey infection;

but they do suggest that very fine particles may also avail to transport influenza virus A. It is hoped to study later whether such fine particles are to be regarded as droplet nuclei in Wells's sense, or as minute fragments of dust.

It is difficult to study the mechanism of cross-infection when the infecting agent will not pass from one animal to another with perfect regularity. It was very encouraging, therefore, when one of our influenza-infected ferrets developed an added streptococcal infection, and thus provided us with a far more reliable method of attacking this problem ; experiments on the combined influenza A and streptococcus infection are described in following papers.

SUMMARY.

Influenza A infection will pass from one ferret to another across a distance of 5 ft. Raising the recipient ferret's cage to a level of 3 ft. above that of the infected animal does not prevent cross-infection. Increased ventilation or the interposition of a screen between the ferrets appear to decrease the chances of cross-infection ; but reasons are given for accepting the results of these tests with reserve. Trials in closed ducts suggest that infection may be conveyed by " droplet nuclei " or other very small particles.

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REFERENCES.

STUART-HARRIS, C. H., ANDREWES, C. H., AND SMITH, W.—(1938) *Med. Res. Council Sp. Rep., Series* No. 228.

WELLS, W. F., AND WELLS, M. W.—(1936) *J. Amer. med. Ass.*, **107**, 1698, 1805.